Evaluation of *Rosa* Species Accessions for Resistance to Eriophyid Mites[1]

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Abstract

Rose rosette disease, caused by rose rosette virus (RRV), is an epidemic affecting nearly every rose cultivar in the United States. The only hosts for *Phyllocoptes fructiphilus*, the eriophyid mite that vectors RRV, are *Rosa* species. Eighteen *Rosa* species were evaluated for mite resistance by collecting foliage samples from July to November in 2016 and 2017, from which mites were extracted. Mites were isolated through a series of sieves and counted using a stereomicroscope. The response variable was expressed as the number of mites per gram of optimal rose tissue. Mite data were evaluated to determine the peak week for mite populations for each year. The mite populations varied by rose species ($\alpha = 0.05$) in 2016 but not 2017. Due to high variability in mite counts, the species were not as clearly distinguishable as expected. This high variability is likely due to factors such as differential growth rates of the roses, weather, presence of RRV in the rose, and the quality of the tissue collected throughout the season. Experimental design revisions are proposed for future studies looking at *Rosa* species resistance to eriophyid mite populations.

Index words: rose rosette virus, rose rosette disease, *Phyllocoptes fructiphilus* Keifer, virus, vector.

Species used in this study: *Phyllocoptes fructiphilus* (Keifer), Prairie Rose [*Rosa arkansana* (Porter), Forest Farm]; Carolina Rose [*Rosa carolina* (L.), Forest Farm]; *Rosa clinophylla* (Thory), Rogue Valley Rose; White Prairie Rose [*Rosa foliolosa* (Nutt.), Rogue Valley Rose]; White Prairie Rose [*Rosa foliolosa* (Nutt.) Antique Rose Emporium]; Father Hugo Rose [*Rosa hugonis*, Rogue Valley Rose]; Musk Rose [*Rosa moschata* (J. Herrm.), Antique Rose Emporium]; Multiflora Rose [*Rosa multiflora* (Thunb.)]; Shining Rose [*Rosa nitida* (Willd.), Rogue Valley Rose]; Shining Rose [*Rosa nitida* (Willd.), Antique Rose Emporium]; Nootka Rose [*Rosa nutkana* (C. Presl.), Rogue Valley Rose]; Tea Rose [*Rosa odorata* (Andrews), Foundation Plant Services, Davis, CA]; Swamp Rose [*Rosa palustris* (Marshall), Antique Rose Emporium]; Swamp Rose [*Rosa palustris* (Marshall), Ever Blooming Antique Rose Emporium]; Chestnut Rose [*Rosa roxburghii* (Tratt.), Antique Rose Emporium]; 'Plena' Chestnut Rose [*Rosa roxburghii* (Tratt.), Rogue Valley Rose]; Rugosa Rose [*Rosa rugosa* (Thunb.), Bailey's Nursery]; 'Alba' Rugosa Rose [*Rosa rugosa* (Thunb.), Bailey's Nursery]; Climbing Prairie Rose [*Rosa setigera* (Michx.), Antique Rose Emporium]; *Rosa soulieana* (Crép.), Ralph Moore; Virginia Rose [*Rosa virginiana* (Mill.), Forest Farm]; Porterfolia Memorial Rose [*Rosa wichuraiana* (Crép.), Antique Rose Emporium]; Mountain Woods' Rose *[Rosa woodsii* (Lindl.), Rogue Valley Rose].

Significance to the Horticulture Industry

Rose rosette virus (RRV) has destroyed many roses and is vectored by the eriophyid mite, *Phyllocoptes fructiphilus*. This mite feeds only on *Rosa* species. Efforts have been made to screen roses for virus resistance, create new rose crosses to develop resistant roses, and develop disease management procedures, but looking for resistance in the vector/ host relationship has remained uninvestigated (Byrne et al. 2019). This study evaluated 18 *Rosa* species, plus multiple accessions of *R. foliolosa, R. nitida, R. palustris, R. roxburghii,* and *R. rugosa*, for resistance to eriophyid mite populations under field conditions. The data showed large variation in mite numbers and were not statistically different due to a relatively small sample size per rose species, environmental conditions, and possible design issues in the experiment. This paper outlines those issues and suggests a plan that may more accurately identify whether resistance to the mite exists in *Rosa* species. Identification of *Rosa* species resistant to eriophyid mite populations would be useful for developing roses resistant to the vector of RRV.

Introduction

Most eriophyid mites that live on roses do not cause significant damage to their host. However, mites will consume nutrients, reduce gas exchange, impact photosynthesis, kill epidermal cells, and in some cases cause deformation of host plant tissues (Sabelis and Bruin 1996). In the United States, there are six known eriophyid mite species that live on roses. They include: *Phyllocoptes fructiphilus, P. adalius, P. linegranulatus, P. chorites, Callyntrotus schlechtendali* (Baker et al. 1996, Keifer 1939a, b, 1940, 1972, Styer 1974, Otero-Colina et al. 2018) and *Eriophyes eremus* (Otero-Colina et al. 2018). These mites have been found on all types of roses including native, naturalized, and ornamental cultivars. Both *P. fructiphilus* and *E. eremus* are refuge-seeking mites, which avoid adverse environmental elements and predatory mites by using micro-environments, such as petiole stem

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interfaces, under sepal trichomes, young folded leaves, and flower buds. All other rose mites in the U.S. are considered vagrant mites, often found crawling on leaf surfaces.

Phyllocoptes fructiphilus is the most economically important eriophyid mite on roses, as it is the vector of rose rosette virus (RRV) (Allington et al. 1968, Amrine et al. 1988, Gergerich and Kim 1983). The mite passively floats on the wind to new hosts in a process called ballooning. Rose rosette virus can be transmitted by stem grafts (Amrine et al. 1988, Gergerich and Kim 1983) but eriophyid vector transmission remains to be the only natural, long distance mode of transmission for RRV. There are no chemical controls available for RRV on the market, therefore control efforts must target the mite vector or resistance to the virus.

Rosa species may vary in hospitality to the eriophyid mites based on the number of niches available, quality of nutrients available, or the presence of phytochemicals that affect their preference, feeding, or growth. In cases in which eriophyid mite populations remain low, it is assumed that the host plant is exhibiting a level of resistance. The objective of this experiment was to determine if the eriophyid mite populations differ among rose species accessions as determined from mite field counts.

Materials and Methods

Plant species. Roses were located at the University of Tennessee Plateau AgResearch and Education Center in Crossville, Tennessee. The roses were planted one year before the study began. Eighteen *Rosa* species, including two accessions for *R. foliolosa, R. nitida, R. roxburghii,* and *R. rugosa* and three accessions for *R. palustris* (total of 24 genotypes) were evaluated for mite resistance using a complete random design with five replicates in one plot. Destructive sampling was used to collect several canes approximately 50 cm (20 in) long from each plant. Sampling occurred biweekly in 2016 and monthly in 2017, starting in July and ending in October. Stem samples were given floral cuts (basal cut performed underwater to reduce air embolisms) to transport samples from the field to the lab.

Mite extraction. From each plant, 10 g (0.4 oz) of optimal rose tissue (preferred niches by *P. fructiphilus* such as petiole stem interfaces, under sepal trichomes, young folded leaves, and flower buds) were collected from the 50 cm stems. Sample tissue was submerged in approximately 250 ml (8.5 fl oz) of Clorox Regular Bleach$_1$ (Oakland, CA)/ Dawn Dish Soap (Cincinnati, OH) dilution (187.5 ml water, 62.5 ml Clorox Regular Bleach$_1$, 4 drops Dawn Dish Soap) and stirred for a maximum of ten min (Monfreda et al. 2007). The tissue solution was poured through a series of sieves: numbers 80, 270, and 500 which had openings of 180, 53, and 25 µm, respectively (Hogentogler, Columbia, Maryland). Contents in the 500 mesh were rinsed with water into a square 100 mm x 100 mm x 15 mm square Petri dish with a 36 square grid (Fisher Scientific, Waltham, Massachusetts).

Counting procedure. Five squares were counted using a 50x stereomicroscope. Averages were calculated for each plate and converted to average number of mites per gram of optimal plant tissue.

Statistical analysis. A generalized linear mixed model analysis of variance (ANOVA) was developed to test the effect of species on the number of mites per gram of plant tissue on each year separately. The number of mites per gram of rose tissue was log transformed to achieve assumptions of ANOVA, including normality of residuals and equal variance, and statistical models were developed using the GLIMMIX procedure in SAS 9.4. Transformed least square means were compared using mean separations, and Tukey's adjustment for multiple comparisons was used to decrease the chance of Type I error probabilities. Statistical significance was determined at $\alpha = 0.05$. Untransformed least square means and standard errors are reported.

Results and Discussion

This study was designed to analyze eriophyid mite preference for different *Rosa* species. After two years of observations, expectations were to identify rose species that exhibited low eriophyid mite populations that may possess some level of mite resistance. Currently, resistant cultivars and therapeutic measures for rose rosette disease (RRD) control are not available. Management strategies are aimed at reducing the spread of the virus by early detection and control of *P. fructiphilus*. Roses with small residential eriophyid mite numbers may be used in the future for breeding eriophyid mite resistance into new rose cultivars. Limiting the eriophyid mite's ability to inhabit roses may limit the spread of RRV by reducing the number of mites available for ballooning. In this study, rose species showed very little statistical difference in the number of mites recovered.

Fixed effect of species. The mite populations (mites per g rose tissue) differed among rose species accessions in 2016 ($P < 0.0001$); however, in 2017 there were no differences between species in mite populations ($P = 0.35$).

Mean separation. Resulting mites per gram least square means and standard errors were compared between *Rosa* species in 2016 and in 2017 (Fig. 1). In 2016, there were a few statistical differences between rose species regarding the number of mites found per gram of tissue. In 2017, no rose species was found to be statistically different in terms of the number of mites per gram. Overall, the number of mites per gram on *Rosa* species in 2016 was 166.9 mites per gram while in 2017 only 8.5 mites were found per gram of rose tissue.

Bleach/ soap solution. At the beginning of the 2017 season, mites were extracted in the same manner as in 2016. New Clorox bleach was purchased and the bottle of dish soap that was used in 2016 was used at the beginning of the 2017 collection season. Mites were impossible to count due to cloudiness of the extraction solution in mite samples and a white precipitant that formed on the bottom

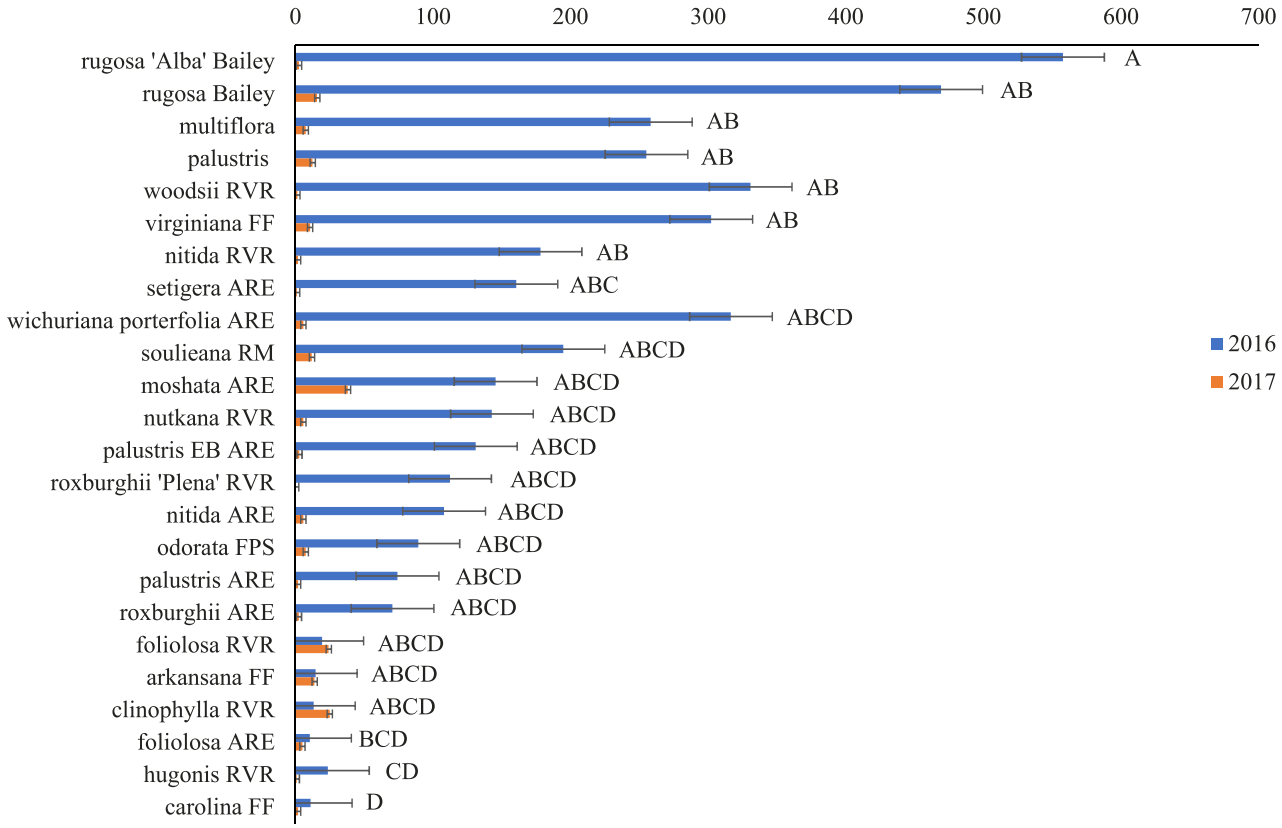

Fig. 1. Log transformed least squares means, standard error, and letter group of the number of *Phyllocoptes fructiphilus* per gram on *Rosa* species in 2016 and 2017 in Tennessee. Letter groupings on the untransformed least squares means were adjusted using Tukey's adjustment and denote statistically similar mite per gram of tissue population levels for the 2016 data. There was no statistical difference in the 2017 data. Statistical significance was determined at α = 0.05. ARE = Antique Rose Emporium, Bailey = Bailey's Nursery, EB = Ever Blooming, FF = Forest Farm, FPS = Foundation Plant Services, Davis CA, RM = Ralph Moore, RVR = Rogue Valley Rose.

of counting plates. After investigating possible errors in procedures, it was determined after contacting the Clorox Company that the formulation of Clorox$_1$ had changed from a sodium chloride (NaClO) concentration of 8.25% to 6.0% and was now being denoted as Clorox$_2$. Using Clorox$_1$ (8.25% NaClO concentration) prevented the cloudiness in the extraction solution. It is recommended that extractions are carried out with Clorox$_1$. If Clorox$_1$ is not available, generic bleach with an 8.25% NaClO concentration probably can be substituted. It was also discovered that the dish soap used from 2016 had spoiled. When added to the bleach solution, the lipids in the 2016 dish soap congealed, creating the white precipitant found in the bottom of the counting plates. It is suggested that a new bottle of dish soap be purchased each year. Fresh extraction solution was made with new soap and bleach and the 2017 mite extractions and counting were conducted with no further issue.

Table 1. Rainfall totals (cm) and monthly high and low temperatures (C) for Crossville, TN in 2016 and 2017 during eriophyid mite sampling from *Rosa* species[z].

Month	Parameter	2016	Normal	2017
July	Rainfall Total	14.38	13.11	10.21
	Avg. High Temp.	29.9	28.9	28.8
	Avg. Low Temp.	19.5	18.3	18.1
August	Rainfall Total	12.32	10.11	10.49
	Avg. High Temp.	29.7	28.3	27
	Avg. Low Temp.	19.3	17.8	16.9
September	Rainfall Total	2.11	9.91	11.08
	Avg. High Temp.	28.3	25	24.4
	Avg. Low Temp.	14.9	13.9	12.7
October	Rainfall Total	0.69	7.70	15.82
	Avg. High Temp.	24.1	20	20.8
	Avg. Low Temp.	9.9	7.8	8.2

[z]Taken from https://www.usclimatedata.com/climate/crossville/tennessee/united-states/ustn0122/2016/10.

Weather factors on population counts. In 2016, the numerical mite counts were significantly higher than the counts from 2017 and weather in Crossville, TN, during the sampling dates, was much warmer and drier in late summer and fall 2016 (Table 1). Eriophyid mite populations have been reported to increase as temperatures increased during the growing season (Easterbook 1978 and 1979, Kozlowski and Boczek 1989, Nault and Styer 1969). Wet foliage may not be as conducive of an environment for mite fecundity. Muraleedharan et al. (1988) found that eriophyid mite populations on tea (*Camellia sinensis*) declined in periods of high rainfall. Additional years of data collection will be necessary to determine if weather patterns, specifically rainfall, play a role in eriophyid mite populations on roses.

Factors contributing to high variation within the data. Although our data identified *Rosa* species that may have some level of resistance to eriophyid mites, year to year eriophyid populations were significantly different. This

suggests that certain years may advance the spread of RRV more than others due to higher eriophyid mite populations. Factors associated with mite migration from one rose host to another are currently unknown but may include temperature, humidity, approaching low pressure areas, strong winds, or changes in the host plant's condition (Michalska et al. 2010). Mite populations on a single host may also play a role in intiated migration to new hosts. High mite populations and environmental factors conducive for infection may lead to high infection rates of RRV.

Other factors that contributed to a lack of statistical differences of mite populations on *Rosa* species may have included differential rates of rose plant growth, symptomatic infection with RRV, and tolerance to RRD. Mite populations are greatest on new succulent plant tissues (Amrine 1996, Amrine et al. 1988). The quality of the tissue collected monthly from fast growing rose species, such as *Rosa multiflora, R. foliolosa RVR,* and *R. foliolosa* ARE remained uniform. The destructive sampling of slow growing roses like *R. hugonis* RVR *and R. arkansana* FF made the collection of uniform samples for estimating mite populations difficult, as new tissue became scarce as the growing season progressed.

During the second year of the study, many plants began to show symptoms of RRD during the sampling season. Some plant replicates only had RRD symptomatic tissue left to sample whereas other plants of the same species were asymptomatic. RRD symptomatic tissue has been reported to support a 14-fold increase of eriophyid mites on RRD symptomatic foliage of *R. multiflora* (Amrine 1996) and 43-fold increase on RRD symptomatic foliage of Knock Out roses (Solo et al. 2019). It is not known if increases in mite populations would be similar for all *Rosa* species. Large data variances were encountered when some species had mixed foliage (RRD symptomatic and asymptomatic) and these impeded detection of statistical differences between accessions.

Tolerance of *Rosa* species to RRV is variable between species. Some infected species, such as *R. multiflora,* continued to grow rapidly and have little or no mortality for the duration of our study, whereas symptomatic plants of *R. odorata* FPS severely declined or died within a year of developing symptoms.

Revised experimental design. For future studies investigating eriophyid mite resistance within *Rosa* species, several modifications to the experimental design are suggested based on our findings. To reduce the effect of destructive sampling on slow growing *Rosa* species, the number of plants per *Rosa* species should be increased. It is suggested that a randomized complete block design with sampling be implemented in the following fashion. In total, there would be a sample population of 24 plants from each species. There will be three blocks, A, B, and C. Eight plants from each *Rosa* species would be randomly assigned to block A, B, and C. Blocks would be sampled in one-month increments. For example, block A will be sampled in June and September, block B in July and October, block C in August and November. This would allow sufficient time between sampling periods for tissue regrowth. Within a block, there could be eight plants for each species, but only five plants sampled based on availability of tissue and health status. It would be ideal for the plants to last the entire study instead of replacing them at the beginning of each year. Not only would it be more economical to maintain plants from year one, it would also allow the plants to grow and have more foliage to sample, minimizing the risk of over sampling. Additionally, using the same plants throughout the entire study would provide a more realistic picture of eriophyid mite populations throughout the growing season. Roses kept from year to year may have overwintering mites which could impact populations sooner than if populations rely on ballooning mites. It is likely that replacement roses have been treated with pesticides to control mites or even come from a location with different eriophyid mite ecology. For consistency and a more realistic picture of how these organisms interact in single location, sustaining plants through the entire study is key. However, roses with RRV symptoms would be removed from the rose field and replaced before the next test season and symptomatic foliage should not be sampled for mite counts.

It would be tempting to recommend that an eriophyid mite resistance study should be conducted in areas where RRD is not known to occur. However, in a concurrent research study (unpublished), it was found that eriophyid mite populations were extremely low or nonexistent in areas where RRD did not occur on the same genotypes known to harbor high populations of mites where RRD is known to exist. There may be environmental factors such as heat and/or humidity, unknown predators or parasites, or other external factors, that may hinder the development of mite populations on roses. Therefore, it is recommended that eriophyid mite resistance studies be conducted in an environment where mite populations are known to increase rapidly and where RRD is present.

In addition, it will be necessary to set strict cane collection criteria to evaluate canes of uniform quality. Canes should be of similar age and asymptomatic of RRD. Pruners should be sterilized in between rose plants to prevent spreading the virus. Using optimal rose tissue for estimating eriophyid mite populations is very important. The eriophyid mite that transmits RRV is a refuge seeking mite that prefers to congregate in petiole stem interfaces, under septal trichomes, in young folded leaves, and in flower buds, therefore, these preferred niches should be used as the optimal tissue sampled for the extraction of eriophyid mites. *Rosa rugosa* and *R. hugonis* have very different leaflet sizes and by avoiding the flat, mature leaflets as part of the 10 g of tissue used to extract mites, the variance in leaflet size is minimized. Likewise, the rate of growth between different *Rosa* species varies slightly and the availability of flowers or young folded leaves may not all occur at the same time or rate. Therefore, a combination of all the optimal tissues would provide a better conglomerate sample than choosing just one preferred niche to sample across all species.

Finding roses with reduced mite populations may be important for developing further RRV and eriophyid mite management strategies. However, even if resistance does exist in *Rosa* species to eriophyid mites, it does not

necessarily mean that the transmission of RRV will be low or be prevented. A single *P. fructiphilus* mite could transmit RRV to *R. multiflora* (Amrine et al. 1988). Host plant resistance could aid in reducing population densities of mites, especially on a single host plant, which decrease the chances of a ballooning mite landing on a new rose bush.

Although the original design did not yield expected results, valuable information was gained on how to redesign the experiment for evaluating eriophyid mite resistance among *Rosa* species. The suggestions listed above should limit the data variance so that meaningful comparisons of eriophyid mite population estimates between *Rosa* species may be performed.

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